DECLARATION

I, Takahiko MIZOBE, Chartered Patent Attorney of Furuya & Co., located at 6th Floor, 2-17-8, Nihonbashi-Hamacho, Chuo-ku, Tokyo 103-0007, Japan, hereby declare the followings:

1. That I am well acquainted with the Japanese and English languages,
2. That the hereto attached English document is a true, full and correct translation of the Japanese patent application No. 2002-346796 filed on November 29, 2002, the priority document;
3. That I make this solemn declaration conscientiously, believing the same to be true and correct.

on the date of May 19, 2009

By Takahiko MIZOBE

Patent Office

Japanese Government

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Applicant: Eisai Co., Ltd.
MERCIAN CORPORATION

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[Inventor]

[Address or Residence] SUNHILLS MATSUSHIRO 303, 1-14-11, Matsushiro, Tsukuba-shi, Ibaraki

[Name] Akifumi OKUDA

[Inventor]

[Address or Residence] 3-9-7, Misono, Tsukuba-shi, Ibaraki

[Name] Satoshi YAMAMOTO

[Inventor]

[Address or Residence] 5-6-3, Matsushiro, Tsukuba-shi, Ibaraki

[Name] Takashi SAKAI

[Inventor]

[Address or Residence] 2-23-1~408, Nagatakita, Minami-ku, Yokohama-shi, Kanagawa

[Name] Susumu TAKEDA

[Inventor]

[Address or Residence] 2-9-2, Kugenumashinmei, Fujisawa -shi, Kanagawa

[Name] Takashi NAKASHIMA

[Inventor]

[Address or Residence] 1-6-601, Nagataminamidai, Minami-ku, Yokohama-shi, Kanagawa

[Name] Katsura KANEKO

[Inventor]

[Address or Residence] 1-3-7-501, Fujisawa, Fujisawa-shi, Kanagawa

[Name] Tomohiro SAMESHIMA

[Applicant]

[Identification Number] 000000217

[Name] Eisai Co., Ltd.

[Applicant]

[Identification Number] 000001915

[Name] MERCIAN CORPORATION

[Agent]

[Identification Number] 100063897

[Patent Attorney]

[Name] Kaoru FURUYA

[Telephone Number] 03(3663)7808

[Agent]

[Identification Number] 100076680

[Patent Attorney]

[Name] Takahiko MIZOBE

[Agent]

[Identification Number] 100087642

[Patent Attorney]

[Name] Satoshi FURUYA

[Agent]

[Identification Number] 100091845

[Patent Attorney]

[Name] Shinji MOCHIDA

[Agent]

[Identification Number] 100098408

[Patent Attorney]

[Name] Kazumasa YOSHITSUNE

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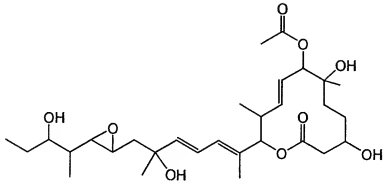
【Designation of the Document】 Specification

【Title of the Invention】 Method of producing macrolide compound

【Patent Claims】

【Claim 1】 A method of producing the macrolide compound 11107D represented by the formula (II):

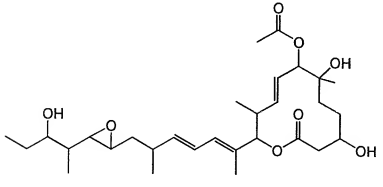
【Chemical Formula 2】



(II)

wherein the macrolide compound 11107D is produced from the macrolide compound 11107B represented by the formula (I):

【Chemical Formula 1】



(I)

by a biological transformation method, which comprises the following processes (A) and (B):

(A) a process of incubating the macrolide compound 11107B

represented by the formula (I) in the presence of a strain having an ability of conducting the above-mentioned biological transformation method and belonging to the genus Streptomyces or a preparation of its cultured mycelia; and

(B) a process of collecting the macrolide compound 11107D represented by the formula (II) from the incubated solution obtained in the process (A).

【Claim 2】 The production method according to claim 1, wherein the strain belonging to the above-mentioned genus Streptomyces is Streptomyces sp. AB-1704 strain (FERM P-18999), A-1544 strain (FERM P-18943) or A-1545 strain (FERM P-18944).

【Claim 3】 Streptomyces sp. AB-1704 strain (FERM P-18999) having the ability of transforming the macrolide compound 11107B represented by the above formula (I) into the macrolide compound 11107D represented by the above formula (II).

【Detailed Description of the Invention】

【0001】

【Technical Field to which the Invention Belongs】

The present invention relates to a method of producing the 12-membered ring macrolide compound 11107D having an antitumor activity by biological transformation and to a novel strain used for it.

【0002】

【Prior Art】

The 12-membered ring macrolide compound 11107D is a 12-membered ring macrolide compound having an excellent antitumor activity and was discovered together with a 11107B substance from a culture product of a Streptomyces sp. Mer-11107

strain (see patent literature 1). The 11107D substance corresponds to a 16-position hydroxide body of the 11107B substance. The productivity thereof is inferior to that of the 11107B substance and it has been therefore desired to establish an efficient production method.

【0003】

【Patent Literature 1】

WO-A02/060890

【0004】

【Problem to be Solved by the Invention】

The purpose of the present invention is to provide a novel method of producing the macrolide compound 11107D by using the macrolide compound 11107B as starting material by a biological transformation method.

【0005】

【Means for Solving the Problem】

The inventors of the present invention have made a trial to screen microorganisms capable of transforming the 16-position hydrogen atom of the macrolide compound 11107B to hydroxyl group from a wide range of microorganism groups to solve the above problem, and as a result, found that a strain belonging to the genus Streptomyces classified into actinomycetes has the above-mentioned transforming function, to complete the present invention.

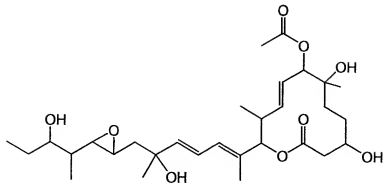
【0006】 - 【0010】

That is, the present invention relates to the following (1) to (3).

(1) A method of producing the macrolide compound 11107D

represented by the formula (II):

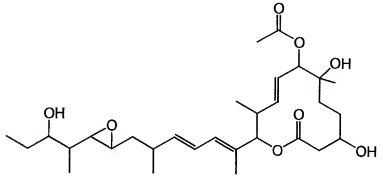
【Chemical Formula 4】



(II)

wherein the macrolide compound 11107D is produced from the macrolide compound 11107B represented by the formula (I):

【Chemical Formula 3】



(I)

by a biological transformation method, which comprises the following processes (A) and (B):

(A) a process of incubating the macrolide compound 11107B represented by the formula (I) in the presence of a strain having an ability of conducting the above-mentioned biological transformation method and belonging to the genus Streptomyces or a preparation of its cultured mycelia; and

(B) a process of collecting the macrolide compound 11107D represented by the formula (II) from the incubated solution obtained in the process (A).

【0011】

(2) The production method according to the above (1), wherein the strain belonging to the above-mentioned genus Streptomyces is Streptomyces sp. AB-1704 strain (FERM P-18999), A-1544 strain (FERM P-18943) or A-1545 strain (FERM P-18944).

【0012】

(3) Streptomyces sp. AB-1704 strain (FERM P-18999) having the ability of transforming the macrolide compound 11107B represented by the above formula (I) into the macrolide compound 11107D represented by the above formula (II).

【0013】

【Mode for Carrying Out the Invention】

In the biological transformation method of the present invention, any microorganisms belonging to the genus Streptomyces may be used regardless of the type of species and strain insofar as it has the ability to transform the macrolide compound 11107B represented by the above formula (I) into the macrolide compound 11107D represented by the above formula (II). However, as the preferable microorganisms, the Streptomyces sp. AB-1704 strain, A-1544 strain and A-1545 strain, each has isolated from the soil.

【0014】

The Streptomyces sp. AB-1704 strain was deposited at International Patent Organism Depositary National Institute of Advanced Industrial Science and Technology as Streptomyces sp.

AB-1704 as of September 5, 2002 (accession number FERM P-18999). Further, the A-1544 strain and A-1545 strain were also deposited at International Patent Organism Depositary National Institute of Advanced Industrial Science and Technology as A-1544 and A-1545 as of July 23, 2002 respectively (accession number FERM P-18943 and FERM P-18944).

【0015】

The taxonomical properties of the above-mentioned strains are as follows.

(The taxonomical properties of the AB-1704 strain)

(1) Morphological characteristics

Rectiflexibiles type aerial hyphae were extended from vegetative hyphae. Spore chains consisting of about 20 to 50 of cylindrical spores were formed at the end of the matured aerial hyphae. The size of the spores was about 0.6 to 0.8 × 1.0 to 1.1 μm, the surface of the spores was smooth, and specific organs such as sporangium, sclerotium and flagellum were not observed.

【0016】

(2) Cultural characteristics on various media

Cultural characteristics of the strain after incubation at 28°C for two weeks on various media are shown in Table 1. The color tone is described by the color names and codes which are shown in the parentheses of the Color Harmony Manual (Container Corporation of America).

【0017】

【Table 1】

Medium	Growth	Aerial hyphae	Color of vegetative hyphae	Soluble pigment
Yeast extract - malt extract agar (ISP-2)	Good	Thick Ivory (2db)	Nude tan (4gc)	None
Oatmeal agar (ISP-3)	Good	Abundant Ivory (2db)	Light melon yellow (3ea)	None
Inorganic salts - starch agar (ISP-4)	Good	Thick Putty - Ivory (1 1/2ec - 2db)	Cork tan (4ie)	None
Glycerol - asparagine agar (ISP-5)	Good	Thick Parchment (1 1/2db)	Nude tan (4gc)	None
Peptone-yeast extract - iron agar (ISP-6)	Good	Abundant White (a)	Light melon yellow (3ea)	None
Tyrosine agar (ISP-7)	Good	Thick Ivory (2db)	Nude tan (4gc)	None

【0018】

(3) Utilization of various carbon sources

Various carbon sources were added to Pridham-Gottlieb agar and cultured at 28°C for 2 weeks. The growth is shown in Table 2.

【0019】

【Table 2】

D-glucose	+	inositol	-
L-arabinose	±	L-rhamnose	+
D-xylose	+	D-mannitol	+
D-fructose	+	D-raffinose	-
sucrose	±		

(+: positive, ±: slightly positive, -: negative)

【0020】

(4) Various physiological properties

Various physiological properties of the present strain are as follows.

(a) Range of growth temperature (yeast extract-malt extract agar, incubation for 2 weeks) 5°C to 33°C

(b) Range of optimum growth temperature (yeast extract-malt extract agar, incubation for 2 weeks) 15°C to 33°C

(c) Liquefaction of gelatin (glucose-peptone-gelatin medium) positive

(d) Coagulation of milk (skim milk medium) positive

(e) Peptonization of milk (skim milk medium) positive

(f) Hydrolysis of starch (inorganic salts-starch agar) positive

(g) Formation of melanoid pigment (peptone-yeast extract-iron agar) negative, (tyrosine agar) negative

(h) Production of hydrogen sulfide (peptone-yeast extract-iron agar) negative

(i) Reduction of nitrate (broth containing 0.1% potassium nitrate) positive

(j) Sodium chloride tolerance (yeast extract-malt extract agar, incubation for 2 weeks) grown at a salt content of 7% or less

【0021】

(5) Chemotaxonomy

LL-diaminopimelic acid was detected from the cell wall of the present strain.

From the above-mentioned microbial characteristics, the present inventors determined that the present strain belongs to the genus Streptomyces.

【0022】

(The taxonomical properties of A-1544 strain)

(1) Morphological characteristics

Spira type aerial hyphae were extended from vegetative hyphae in this strain. Spore chains consisting of about 10 to 20 of cylindrical spores were formed at the end of the matured aerial hyphae. The size of the spores was about 1.0 × 1.2 to 1.4 μm, the surface of the spores was spiny, and specific organs such as sporangium, sclerotium and flagellum were not observed.

【0023】

(2) Cultural characteristics on various media

Cultural characteristics of the strain after incubation at 28°C for two weeks on various media are shown in Table 3. The color tone is described by the color names and codes which are shown in the parentheses of the Color Harmony Manual (Container Corporation of America).

【0024】

【Table 3】

Medium	Growth	Aerial hyphae	Color of vegetative hyphae	Soluble pigment
Yeast extract - malt extract agar (ISP-2)	Good	Thick Silver gray (3fe)	Light melon yellow (3ea)	None
Oatmeal agar (ISP-3)	Good	Abundant Light gray - Silver gray (d - 3fe)	Light melon yellow (3ea)	None
Inorganic salts - starch agar (ISP-4)	Good	Abundant Silver gray (3fe)	Light melon yellow (3ea)	None
Glycerol - asparagine agar (ISP-5)	Good	Abundant Ashes (5fe)	Light melon yellow (3ea)	None
Peptone-yeast extract - iron agar (ISP-6)	Good	None	Light melon yellow (3ea)	Pale blackish brown
Tyrosine agar (ISP-7)	Good	Abundant Covert gray (2fe)	Light melon yellow (3ea)	None

【0025】

(3) Utilization of various carbon sources

Various carbon sources were added to Pridham-Gottlieb agar and cultured at 28°C for 2 weeks. The growth is shown in Table 4.

【0026】

【Table 4】

D-glucose	+	inositol	-
L-arabinose	+	L-rhamnose	+
D-xylose	+	D-mannitol	+
D-fructose	+	D-raffinose	-
sucrose	±		

(+: positive, ±: slightly positive, -: negative)

【0027】

(4) Various physiological properties

Various physiological properties of the present strain are as follows.

(a) Range of growth temperature (yeast extract-malt extract agar, incubation for 2 weeks) 15°C to 41°C

(b) Range of optimum growth temperature (yeast extract-malt extract agar, incubation for 2 weeks) 20°C to 37°C

(c) Liquefaction of gelatin (glucose-peptone-gelatin medium) positive

(d) Coagulation of milk (skim milk medium) positive

(e) Peptonization of milk (skim milk medium) positive

(f) Hydrolysis of starch (inorganic salts-starch agar) positive

(g) Formation of melanoid pigment (peptone-yeast extract-iron agar) positive, (tyrosine agar) negative

(h) Production of hydrogen sulfide (peptone-yeast extract-iron agar) positive

(i) Reduction of nitrate (broth containing 0.1% potassium nitrate) negative

(j) Sodium chloride tolerance (yeast extract-malt extract agar, incubation for 2 weeks) grown at a salt content of 7% or less

【0028】

(5) Chemotaxonomy

LL-diaminopimelic acid was detected from the cell wall of the present strain.

From the above-mentioned microbial characteristics, the present inventors determined that the present strain belongs to the genus Streptomyces.

【0029】

(The taxonomical properties of A-1545 strain)

(1) Morphological characteristics

Rectiflexibiles type aerial hyphae were extended from vegetative hyphae in this strain. Spore chains consisting of about 50 of spores were formed at the end of the matured aerial hyphae. The size of the spores was about $0.8 \times 1.0\ \mu m$, the surface of the spores was smooth, and specific organs such as sporangium, sclerotium and flagellum were not observed.

【0030】

(2) Cultural characteristics on various media

Cultural characteristics of the strain after incubation at 28°C for two weeks on various media are shown in Table 5. The color tone is described by the color names and codes which are shown in the parentheses of the Color Harmony Manual (Container Corporation of America).

【0031】

【Table 5】

Medium	Growth	Aerial hyphae	Color of vegetative hyphae	Soluble pigment
Yeast extract - malt extract agar (ISP-2)	Good	Abundant Grayish yellowish pink (5cb)	Light melon yellow - Nude tan (3ea - 4gc)	None
Oatmeal agar (ISP-3)	Moderate	Thin Grayish yellowish pink (5cb)	Pearl pink (3ca)	None
Inorganic salts - starch agar (ISP-4)	Good	Thin Grayish yellowish pink (5cb)	Light Ivory (2ca)	None
Glycerol - asparagine agar (ISP-5)	Good	Abundant Grayish yellowish pink (5cb)	Pearl pink (3ca)	None
Peptone-yeast extract - iron agar (ISP-6)	Moderate	None	Light melon yellow (3ea)	None
Tyrosine agar (ISP-7)	Good	Abundant Grayish yellowish pink (5cb)	Light melon yellow (3ea)	None

【0032】

(3) Utilization of various carbon sources

Various carbon sources were added to Pridham-Gottlieb agar and cultured at 28°C for 2 weeks. The growth of the strain is shown in Table 6.

【0033】

【Table 6】

D-glucose	+	inositol	±
L-arabinose	+	L-rhamnose	+
D-xylose	+	D-mannitol	+
D-fructose	+	D-raffinose	+
sucrose	–		

(+: positive, ±: slightly positive, -: negative)

【0034】

(4) Various physiological properties

Various physiological properties of the present strain are as follows.

(a) Range of growth temperature (yeast extract-malt extract agar, incubation for 2 weeks) 10°C to 37°C

(b) Range of optimum growth temperature (yeast extract-malt extract agar, incubation for 2 weeks) 20°C to 33°C

(c) Liquefaction of gelatin (glucose-peptone-gelatin medium) negative

(d) Coagulation of milk (skim milk medium) positive

(e) Peptonization of milk (skim milk medium) positive

(f) Hydrolysis of starch (inorganic salts-starch agar) positive

(g) Formation of melanoid pigment (peptone-yeast extract-iron agar) negative, (tyrosine agar) negative

(h) Production of hydrogen sulfide (peptone-yeast extract-iron agar) positive

(i) Reduction of nitrate (broth containing 0.1% potassium nitrate) negative

(j) Sodium chloride tolerance (yeast extract-malt extract agar, incubation for 2 weeks) grown at a salt content of 7% or less

【0035】

(5) Chemotaxonomy

LL-diaminopimelic acid was detected from the cell wall of the present strain.

From the above-mentioned microbial characteristics, the present inventors determined that the present strain belongs to the genus Streptomyces.

【0036】

According to the present invention, first in the process (A), the macrolide compound 11107B which is starting material (substrate) is incubated in the presence of the above strains or products prepared by its cultured mycelia and further in the presence of oxygen. This treatment may be carried out by adding the substrate in the culture broth when culturing the above strains in an aerobic condition or, as the case may be, by adding the substrate in a suspension solution of the cultured mycelia of the above strains as it is or of a product prepared by homogenizing these cells with flowing gas containing oxygen, for example, air.

【0037】

The substrate may be added to the culture broth before culturing or when a fixed time passes after the start of culturing. The above mycelia may be produced by inoculating any one of the above strains into a medium containing a nutrient and culturing aerobically. The culturing of the strain for producing mycelia preparations or the culturing of the strain which is carried out in the situation where the substrate is added may be performed according to a method of culturing general microorganisms fundamentally. However, in general, the culturing is preferably carried out in an aerobic condition by, for example, flask shaking culture and tank culture according to liquid culture.

【0038】

As the medium used for culturing, any medium may be used insofar as it contains a nutrient which microorganisms belonging

to the genus Streptomyces can utilize, and various synthetic media, semi-synthetic media and natural media may be all utilized. As the medium composition, various carbon sources such as glucose, galactose, sucrose, maltose, fructose, glycerin, dextrin, starch, molasses and soybean oil may be used either independently or in combinations.

【0039】

As the nitrogen source, there can be used a single or a combination of organic nitrogen sources such as pharma media, peptone, meat extract, soybean meal, fish meal, gluten meal, casein, dry yeast, amino acid, yeast extract, NZ-case and urea, and inorganic nitrogen sources such as sodium nitrate and ammonium sulfate. Additionally, for example, there can be added and used salts such as sodium chloride, potassium chloride, calcium carbonate, magnesium sulfate, sodium phosphate, potassium phosphate copper sulfate, iron sulfate, manganese chloride or cobalt chloride; heavy metal salts; vitamins such as vitamin B or biotin; and inclusion agents such as cyclodextrins, if necessary. Further, when foaming is remarkable during culture, various defoaming agents can be appropriately added in the medium as necessary. When the defoaming agent is added, it is required to set at a concentration for not adversely affecting the production of an objective substance.

【0040】

The culture condition can be appropriately selected within the range at which the microbial strain grows well and can produce the above-mentioned substance. For example, the pH of a medium is about 5 to 9, and preferably nearby neutral in general. The

temperature of fermentation is usually kept at 20°C to 40°C and preferably 24°C to 30°C. The fermentation period is about 1 to 8 days, and usually about 2 to 5 days. The above-mentioned fermentation conditions can be suitably changed in accordance with the kind and property of microorganism used, external conditions and the like, and it is needless to say that an optimum condition can be selected.

【0041】

Also, the culture mycelia preparation is prepared by suspending mycelia separated by centrifugation or filtration or homogenized in a proper solution after the culturing is finished. Examples of the solution used for the suspension of the mycelia include the above-mentioned medium or buffer solutions such as tris-acetic acid, tris-hydrochloric acid, sodium succinate, sodium citrate, sodium phosphate and potassium phosphate either singly or in combinations. The pH of the buffer solution is 5.0 to 9.0 and preferably 6.0 to 7.5.

【0042】

The 11107B substance as the substrate may be added in a culture broth or a suspension solution of mycelia either as a powder as it is or as a solution dissolved in a water-soluble solvent, for example, ethanol. The amount of the 11107B substance added is preferably 50 to 2000 mg per 1 L of the culture broth in the case of the culture broth. After the addition of the substrate, procedures such as flask shaking or tank culture are conducted at 27 to 31°C for about 1 to 3 days to run a reaction in an aerobic condition, whereby the 11107B substance as the substrate can be converted into the 11107D substance.

【0043】

Next, in the process (B), the target 11107D substance is recovered from the incubated solution obtained in the above process (A). Suitable methods are selected from various known purification methods which are usually used to isolate microorganism metabolites and used in combination to isolate the 11107D substance from the reaction mixture in the process (A). For example, extraction by an organic solvent such as methanol, ethanol, butanol, acetone, ethyl acetate, butyl acetate, chloroform or toluene; various kinds of ion-exchange chromatography; gel filtration chromatography using Sephadex LH-20; the treatment of adsorption and desorption by absorption chromatography using a hydrophobic adsorbing resin such as Diaion HP-20, active carbon or silica gel, or thin layer chromatography; or high-performance liquid chromatography using a reverse phase column and so on may be used independently or in combinations or used repeatedly whereby the 11107D substance can be separated and purified.

【0044】

【Examples】

Hereinafter, the present invention will be explained in more detail by way of Examples, which are not intended to limit the scope of the present invention. In the following Examples, all designations of percentage (%) indicate weight percentage (% (w/v)), unless otherwise noted.

【0045】

Referential Example 1 (Production of 11107B substance as starting material)

One loopful of the slant culture (ISP-2 medium) of Streptomyces sp. Mer-11107 strain (FERM BP-7812) was inoculated into a 500 mL Erlenmeyer flask containing 50 mL of seed medium (2.0% of glucose, 1.0% of ESUSAN-MEAT (manufactured by Ajinomoto Co. Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), 0.25% of sodium chloride, 0.32% of calcium carbonate, pH 6.8 before sterilized), and it was cultured at 28°C for two days to give the first seed culture broth. 0.1 mL of the culture broth was inoculated into a 500 mL Erlenmeyer flask containing 100 mL of the same seed medium and it was cultured at 28°C for one day to give the second seed culture broth. The second seed culture broth (800 mL) thus obtained was inoculated into a 200 L tank containing 100 L of a production medium (5.0% of soluble starch, 0.8% of Pharmamedia, 0.8% of gluten meal, 0.5% of yeast extract and 0.1% of calcium carbonate, pH 6.8 before sterilized) and it was cultured for five days with flowing air and stirring in the condition: culture temperature: 28°C, rotation: 90 rpm, amount of air: 1.0 vvm and internal pressure: 20 kPa, to give a culture broth.

【0046】

A part of the culture broth (10 L) thus obtained was extracted with 10 L of 1-butanol, and then the resulting butanol layer was evaporated to dryness, to give 100 g of crude active fraction. The crude active fraction was applied on Sephadex LH-20 (1500 mL; manufactured by Pharmacia Co. Ltd.), and eluted with tetrahydrofuran-methanol (1:1) as a solvent. An eluted fraction from 540 mL to 660 mL was concentrated to dryness, to give a residue (660 mg). The resulting residue was dissolved

in a mixture of ethyl acetate and methanol (9:1; v/v) and subjected to silica gel column chromatography (WAKO GEL C-200, 50 g). The column was eluted with a mixture (2 L) consisting of n-hexane and ethyl acetate (1:9, v/v), the fractions eluted from 468 mL to 1260 mL were collected, evaporated to give 25 mg of a crude active fraction.

【0047】

The obtained crude active fraction was subjected to preparative high performance liquid chromatography (HPLC) under the following preparative HPLC condition (A), and the fractions eluted at the retention time of 34 minutes were collected. After removing acetonitrile, the respective fractions were desalted by HPLC under the following preparative HPLC condition (B) to give 11107B (Retention time: 37 minutes, 6 mg).

【0048】

Preparative HPLC conditions A:

Column: YMC-PACK ODS-AM SH-343-5AM, φ20 mm × 250 mm (manufactured by YMC Co.)

Temperature: room temperature

Flow rate: 10 mL/min.

Detection: 240 nm

Eluent: acetonitrile/0.15% aqueous potassium dihydrogenphosphate (pH 3.5) (2:8 to 8:2, v/v, 0 to 50 min., linear gradient)

【0049】

Preparative HPLC conditions B:

Column: YMC-PACK ODS-AM SH-343-5AM, φ20 mm × 250 mm (manufactured by YMC Co.)

Temperature: room temperature

Flow rate: 10 mL/min.

Detection: 240 nm

Eluent: methanol/water (2:8 to 10:0, v/v, 0 to 40 min., linear gradient)

【0050】

Example 1 Conversion by AB-1704 strain in a test tube scale

One loopful of the slant culture (0.5% of soluble starch, 0.5% of glucose, 0.1% of fish meat extract (manufactured by Wako Pure Chemical Industries, Ltd.), 0.1% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), 0.2% of NZ-case (manufacured by Humko Sheffield Chemical Co.), 0.2% of sodium chloride, 0.1% of calcium carbonate and 1.6% of agar (manufactured by Wako Pure Chemical Industries, Ltd.)) of Streptomyces sp. AB-1704 strain (FERM P-18999) isolated from the soil was inoculated into a 65 mL test tube containing 7 mL of a seed medium (2.0% of soluble starch, 1.0% of glucose, 0.5% of polypeptone (manufactured by Nihon Pharmaceutical Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.) and 0.1% of calcium carbonate), and it was cultured at 28°C for three days in a rotary shaker to give a seed culture broth.

【0051】

Further, 0.5 mL of the seed culture broth was inoculated into a 65 mL test tube containing 7 mL of a production medium (2.0% of soluble starch, 1.0% of glucose, 0.5% of polypeptone (manufactured by Nihon Pharmaceutical Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), and 0.1% of calcium carbonate), and it was cultured at 28°C for three

days in a rotary shaker. Next, a 25 mg/mL solution of the substrate 11107B substance in ethanol was prepared, and 0.2 mL thereof was added to the culture. After addition, it was shaken at 28°C for 48 hours to carry out conversion reaction. After the reaction, it was analyzed by HPLC under the following analytic HPLC condition (a) to confirm the formation of the 11107D substance in the reaction mixture.

【0052】

Analytic HPLC condition (a)

Column: CAPCELL PAK C18 SG120 φ4.6 mm × 250 mm (manufactured by SHISEIDO CO.,)

Temperature: 40°C

Flow rate: 1 mL/min.

Detection: 240 nm

Eluent: acetonitrile/0.15% potassium dihydrogenphosphate (pH 3.5) (3:7 to 5:5 , v/v, 0 to 18 minutes, linear gradient), acetonitrile/0.15% potassium dihydrogenphosphate (pH 3.5) (5:5 to 85:15, v/v, 18 to 22 minutes, linear gradient)

Retention time: 11107D substance 9.9 min., 11107B substance 19.4 min.

【0053】

Example 2 Conversion by A-1545 strain in a test tube scale

One loopful of the slant culture (yeast-malt agar) of A-1545 strain (FERM P-18944) isolated from the soil was inoculated into a 250 mL Erlenmeyer flask containing 20 mL of a seed medium (2.4% of soluble starch, 0.1% of glucose, 0.5% of soybean meal (ESUSAN-MEAT manufactured by Ajinomoto Co., Ltd.), 0.3% of beef extract (manufactured by Difco), 0.5% of yeast extract

(manufactured by Difco), 0.5% of triptone-peptone (manufactured by Difco), and 0.4% of calcium carbonate), and it was cultured at 28°C for three days in a rotary shaker to give a seed culture broth.

【0054】

Further, 0.6 mL of the seed culture broth was inoculated into a 500 mL Erlenmeyer flask containing 60 mL of a production medium (2% of soluble starch, 2% of glucose, 2% of soybean meal (ESUSAN-MEAT manufactured by Ajinomoto Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), 0.25% of sodium chloride, 0.32% of calcium carbonate, 0.0005% of copper sulfate, 0.0005% of manganese chloride, 0.0005% of zinc sulfate, pH 7.4 before sterilization), and it was cultured at 28°C for four days in a rotary shaker. Each 2 mL of the resulting culture was dispensed into 15 mL test tubes. Next, a 20 mg/mL solution of the substrate 11107B substance in dimethyl sulfoxide was prepared, and 0.05 mL thereof was added. After the addition, it was shaken at 28°C for 23 hours to carry out conversion. After the reaction, it was analyzed by HPLC under the following analytic HPLC condition (b) to confirm the formation of the 11107D substance in the reaction mixture.

【0055】

Analytic HPLC condition (b)

Column: CAPCELL PAK C18 SG120 φ4.6 mm × 250 mm (manufactured by SHISEIDO CO.,)

Temperature: 40°C

Flow rate: 1 mL/min.

Detection: 240 nm

Eluent: acetonitrile/water (50:50, v/v) Isocratic

Retention time: 11107B substance 7.2 min., 11107D substance 3.6 min.

【0056】

Example 3 Conversion by AB-1704 strain in a flask scale

One loopful of the slant culture (0.5% of soluble starch, 0.5% of glucose, 0.1% of fish meat extract (manufactured by Wako Pure Chemical Industries, Ltd.), 0.1% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), 0.2% of NZ-case (manufacured by Humko Sheffield Chemical Co.), 0.2% of sodium chloride, 0.1% of calcium carbonate, and 1.6% of agar (manufactured by Wako Pure Chemical Industries, Ltd.)) of Streptomyces sp. AB-1704 strain (FERM P-18999) isolated from the soil was inoculated into a 500 mL Erlenmeyer flask containing 100 mL of a seed medium (2.0% of soluble starch, 1.0% of glucose, 0.5% of polypeptone (manufactured by Nihon Pharmaceutical Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.) and 0.1% of calcium carbonate), and it was cultured at 28°C for three days on a rotary shaker to give a seed culture broth. Further, 2 mL of the seed culture broth was inoculated into each of 150 Erlenmeyer flasks having a capacity of 500 mL and containing 100 mL of a production medium (2.0% of soluble starch, 1.0% of glucose, 0.5% of polypeptone (manufactured by Nihon Pharmaceutical Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.) and 0.1% of calcium carbonate), and it was cultured at 28°C for two days on a rotary shaker.

【0057】

A 20 mg/mL solution of the substrate 11107B substance in ethanol was prepared, and each 0.44 mL thereof was added to the resulting culture (100 mL/500 mL Erlenmeyer flask, 150 flasks). After the addition, it was shaken at 28°C for 9 hours to conduct conversion reaction. After the completion of the reaction, the cultures were collected and separated into the culture supernatant and the mycelia by centrifugation at 2700 rpm for 10 minutes. The mycelia was extracted with 5 L of methanol and filtered to give the methanol extract solution. This methanol extract solution was evaporated to remove methanol, combined with the culture supernatant and extracted with 10 L of ethyl acetate. The resulting ethyl acetate solution was evaporated to give 2090 mg of a crude active fraction. The crude active fraction was dissolved in 4 mL of a mixture of tetrahydrofuran-methanol (1:1, v/v) and 6 mL of a 50% aqueous solution of acetonitrile, subjected to ODS column chromatography (manufactured by YMC Co., ODS-AM 120-S50 φ3.6 cm × 43 cm) and eluted with a 40% aqueous solution of acetonitrile. An eluted fraction from 336 mL to 408 mL was concentrated to dryness under reduced pressure to give 560 mg of the residue. Further, the residue was dissolved in 10 mL of a 50% aqueous methanol solution, subjected to ODS column chromatography (manufactured by YMC Co., ODS-AM 120-S50 φ3.6 cm × 40 cm) and eluted with a 50% aqueous solution of methanol. An eluted fraction from 1344 mL to 1824 mL was concentrated to dryness under reduced pressure to give 252 mg of 11107D substance.

【0058】

Example 4 Conversion by A-1545 strain in a flask scale

One loopful of the slant culture (yeast-malt agar) of A-1544 strain (FERM P-18943) was inoculated into a 250 mL Erlenmeyer flask containing 25 mL of a seed medium (2% of soluble starch, 2% of glucose, 2% of soybean meal (ESUSAN-MEAT manufactured by Ajinomoto Co., Ltd.), 0.5% of yeast extract (manufactured by Difco), 0.25% of sodium chloride, and 0.32% of calcium carbonate, pH 7.4 before sterilized), and it was cultured at 28°C for two days in a rotary shaker to give a seed culture broth. Each 0.75 mL of the broth was dispensed into 2 mL serum tubes (manufactured by Sumitomo Bakelite Co., Ltd.), and an equal amount of a 40% aqueous solution of glycerol was added. After stirring, it was frozen at -70°C to give a frozen seed. The frozen seed was melted, 0.25 mL thereof was inoculated into a 250 mL Erlenmeyer flask containing 25 mL of a seed medium (2% of soluble starch, 2% of glucose, 2% of soybean meal (ESUSAN-MEAT manufactured by Ajinomoto Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), 0.25% of sodium chloride and 0.32% of calcium carbonate, pH 7.4 before sterilized), and it was cultured at 28°C for two days on a rotary shaker to give a seed culture broth. Further, the seed culture broth (0.5 mL) was inoculated into a 500 mL Erlenmeyer flask containing 100 mL of a production medium (2% of soluble starch, 2% of glucose, 2% of soybean meal (ESUSAN-MEAT manufactured by Ajinomoto Co., Ltd.), 0.5% of yeast extract (manufactured by Oriental Yeast Co., Ltd.), 0.25% of sodium chloride, and 0.32% of calcium carbonate, pH 7.4 before sterilized), and it was cultured at 28°C for three days on a rotary shaker.

【0059】

Each of the resulting culture broths (100 mL/500 mL Erlenmeyer flask, 10 flasks) was subjected to centrifugation at 3000 rpm for 10 minutes to collect microorganism cells, and they were suspended into 100 mL of a 50 mM phosphate buffer solution (pH 6.0). Next, a 100 mg/mL solution of the substrate 11107B substance in dimethyl sulfoxide was prepared, and each 0.5 mL thereof was added. After the addition, it was shaken at 28°C for 24 hours to conduct conversion reaction. After the completion of the reaction, the reaction solutions were collected and separated into the supernatant and the mycelia by centrifugation at 5000 rpm for 20 minutes. The supernatant was extracted with 1 L of ethyl acetate. The mycelia was extracted with 500 mL of methanol and then filtered to obtain a methanol extract. The methanol extract was evaporated to remove methanol and extracted with 1 L of ethyl acetate. Each of the ethyl acetate layers was washed with water, dried and dehydrated over anhydrous sodium sulfate, and the combined and evaporated to give 937 mg of a crude fraction. The crude fraction was subjected to silica gel column chromatography (Kiesel gel 60, 50 g) and eluted with 1200 mL of a mixture of ethyl acetate and n-hexane (90:10; v/v) to obtain 234 mg of an active fraction. The resulting active fraction was subjected to preparative high performance liquid chromatography (HPLC) under the following preparative HPLC condition (C), and the resulting eluate was analyzed by HPLC under the following analytic HPLC condition (c). The solvent was removed from the fraction containing the 11107D substance thus obtained, to give 80 mg of the 11107D substance.

【0060】

Preparative HPLC condition (C)

Column: CAPCELL PAK C18 UG120 φ30 mm × 250 mm (manufactured by SHISEIDO Co.)

Flow rate: 20 mL/min.

Detection: 240 nm

Eluent: acetonitrile/water (30:70, v/v) isocratic

【0061】

Analytic HPLC condition (c)

Column: CAPCELL PAK C18 SG120 φ4.6 mm × 250 mm (manufactured by SHISEIDO Co.)

Temperature: 40°C

Flow rate: 1 mL/min.

Detection: 240 nm

Eluent: acetonitrile/water (35:65, v/v) isocratic

Retention time: 11107D substance 7.8 min.

【0062】

Example 5 Conversion by A-1545 strain in a flask scale

Each of cultures of A-1545 strain (FERM P-18944) (100 mL/500 mL Erlenmeyer flask, 10 flasks) obtained by a similar method as described in Example 4 was subjected to centrifugation at 3000 rpm for 10 minutes to collect microorganism cells, and they were suspended into 100 mL of a 50 mM phosphate buffer solution (pH 6.0). Next, a 100 mg/mL solution of the substrate 11107B in dimethyl sulfoxide was prepared, and each 1 mL thereof was added. After the addition, it was shaken at 28°C for 24 hours to run a conversion reaction. After the completion of the reaction, the reaction solutions were collected and separated into the supernatant and the mycelia by centrifugation at 5000

rpm for 20 minutes. The supernatant was extracted with 1 L of ethyl acetate. The mycelia was extracted with 500 mL of acetone, and then filtered to give an acetone extract. The acetone extract solution was evaporated to remove acetone, and then it was extracted with 1 L of ethyl acetate. Each of the ethyl acetate layers was washed with water, dried and dehydrated over anhydrous sodium sulfate, and then combined and evaporated to give 945 mg of a crude fraction. The crude fraction was subjected to silica gel column chromatography (Kiesel gel 60, 50 g), eluted with 100 mL of a mixture of ethyl acetate and n-hexane (50:50; v/v), 200 mL of a mixture of ethyl acetate and n-hexane (75:25; v/v), and a mixture (600 mL) of ethyl acetate and n-hexane (90:10; v/v), to give 463 mg of an active fraction. The resulting active fraction was subjected to preparative high performance liquid chromatography (HPLC) under the preparative condition (C) described in Example 4, and the resulting eluate was analyzed by HPLC under the analytic HPLC condition (c) described in Example 4. The solvent was removed from the active fraction containing 11107D substance thus obtained, to give 304 mg of the 11107D substance.

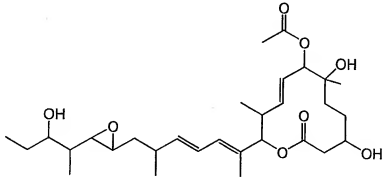
【Designation of the Document】 Abstract

【Abstract】

【Subject】 To provide a novel method of producing the 12-membered ring macrolide compound 11107D having an antitumor activity by biological transformation.

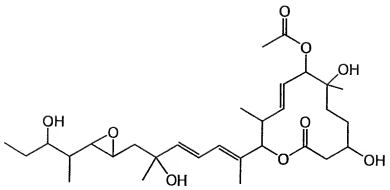
【Means for Solution】 Starting material is incubated in the presence of a strain belonging to the genus Streptomyces (for example, Streptomyces sp. AB-1704 strain (FERM P-18999)), which has the ability of transforming the 12-membered ring macrolide compound 11107B represented by the formula (I), which is the starting material, into a 11107D substance represented by the formula (II), or a preparation of its cultured mycelia and oxygen, and then 11107D substance which is a target material is collected from the treating solution.

【Chemical Formula 1】



(I)

【Chemical Formula 2】



(II)

【Selected Figure】 None.

Particulars of Applicant

Identification Number	[000000217]
1. Date of Changing	August 29, 1990
[Reasons of Changing]	New Registration
Address	6-10, Koishikawa 4-chome, Bunkyo -ku, Tokyo
Name	Eisai Co., Ltd.

Particulars of Applicant

Identification Number	[000001915]
1. Date of Changing	August 8, 1990
[Reasons of Changing]	New Registration
Address	5-8, Kyobashi 1-chome, Chuo--ku, Tokyo
Name	MERCIAN CORPORATION